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High Intensity Interval Training Increases Natural Killer Cell Number and Function in Obese Breast Cancer-challenged Mice and Obese Women

SHORT COMMUNICATION

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High intensity interval training (HIIT) boosts natural killer (NK) cell number and activity in normal weight breast cancer patients; however, whether this occurs in obese individuals is not well established. The goal of this study was to determine whether HIIT effectively boosts NK cells as a therapeutic strategy against breast cancer in an obese mouse model and in overweight/obese women. Diet induced female C57Bl/6 obese mice were assigned to undergo HIIT for four weeks or remain sedentary. Female participants were subjected to a six weeks HIIT protocol. HIIT mice acclimatized to treadmill running were subsequently injected with 5×10^5 polyoma middle T (MT) breast cancer cells intravenously. NK cell number and activation were monitored using flow cytometry, and tumor burden or lipid content evaluated from histological lung and liver tissues, respectively. In both mice and humans, circulating NK cell number and activation (CD3−NK1.1+CD27+ and CD3−CD56+, respectively) markedly increased immediately after HIIT. HIIT obese mice had reduced lung tumor burden compared to controls following MT challenge, and had diminished hepatic lipid deposition despite minimal body weight loss. Our findings demonstrate that HIIT can benefit obese individuals by enhancing NK cell number and activity, reducing tumor burden, and enhancing metabolic health.
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Key Words: High-intensity interval training, Obesity, Natural killer cell activation, Tumor metastasis, Breast cancer

INTRODUCTION

Obesity is a risk factor for various ailments, including breast cancer in women.[1] In pre- and post-menopausal breast cancer patients, obesity is associated with a marked reduction in survival compared to normal weight patients.[2] This increased risk of mortality may result from impaired innate immunity. Innate lymphocytes, specifically natural killer (NK) cells, play a vital role in breast cancer immunosurveillance and exert antitumor responses.[3] Lack of self major histocompatibility complex class I molecules on tumors activate NK cells, inducing cell-death via cytotoxic granule release, death receptor-induced apoptosis, or antibody dependent cellular cytotoxicity.[3,4] Consequently, impaired circulating NK cell activity in obese individuals correlates with increased risk for cancer development.[5] Studies show that body mass index correlates directly with NK cell apoptosis and inversely with circulating NK cell number in women.[6,7] Furthermore, obese individuals have reduced circulating levels of the cytokine interleukin (IL)-15, which is vital to NK cell development.[8] For example, IL-15 deficient mice lack mature NK cells and are highly susceptible to breast cancer metastasis compared to control mice.[9] Therefore, therapeutic strategies boosting innate immunity could improve NK cell immunosurveillance and cytotoxicity in obese individuals susceptible

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to breast cancer.

Endurance exercise is a possible cancer intervention known to increase peripheral NK cell number and activity in normal weight individuals. Of the peripheral blood mononuclear cells (PBMCs), NK cells are the most responsive to exercise.[10] During exercise, circulating epinephrine levels selectively increase and bind β-adrenergic receptors on NK cells, mobilizing cells into the bloodstream.[10,11] Exercise therefore increases NK cell cytotoxicity on a macro level by elevating the total percentage of circulating NK cells.[12] In particular, high intensity interval training (HIIT) results in a greater increase in NK cell count than with moderate intensity exercise.[13] Moreover, a previous study employing aerobic exercise revealed that the increase in NK cell count was 35% greater in females than in males.[14] Given their highly responsive innate immunity, HIIT as an intervention strategy has promising therapeutic potential in female breast cancer patients.

Physical activity can decrease the risk of cancer recurrence and cancer-related deaths by 40% in normal weight female breast cancer patients.[15] However, the therapeutic benefits of exercise in obese patients have not been adequately examined. The aim of our study was to determine whether HIIT is an effective NK cell boosting therapeutic strategy against breast cancer in a diet-induced obese mouse model and in obese women.

MATERIALS AND METHODS

1. Mice and human exercise high intensity interval training protocol

Six-week-old female C57Bl/6 (B6) mice (Charles River Laboratory, Wilmington, MA, USA) were either fed a 60% high fat (Research Diets, New Brunswick, NJ, USA) or chow diet (Envigo, Indianapolis, IN, USA) ad libitum for 15 weeks and housed under controlled conditions (12:12 L:D; 22°C) in McMaster University's Central Animal Facility. High fat fed obese mice were assigned to HIIT or remained sedentary. Mice assigned to HIIT were acclimatized to the treadmill over 3 days, running at 10 to 15 m/min for 15 minutes, as previously described.[16] HIIT involved treadmill running at 15 m/min for 2 minutes followed by 2 minutes of rest for 60 minutes 3 d/wk for 4 weeks. All animal experiments were approved by McMaster University's Animal Research Ethic Board (AREB).

Three overweight/obese women, who took part in this study, were deemed sedentary based on self-reported habitual physical activity (≤2 sessions/week of structured exercise lasting ≤30 minutes) as previously described.[17] The study protocol was approved by the AREB and Faculty of Health Science Research Ethics Board at McMaster University. The experimental HIIT protocol for the human subjects was performed as previously described.[17] Briefly, participants underwent a 6-week HIIT protocol three times per week involving 10 × 60 seconds cycling intervals at 90% maximal heart rate interspersed with 60 seconds of recovery. During the final training session, blood samples were collected immediately pre- and post-HIIT, as well as following 20 minutes of seated rest after exercise.

2. Fluorescence-activated cell sorting analysis

Baseline and post-HIIT blood samples were collected in anticoagulant citrate dextrose solution (BD Diagnostics, Oakville, ON, Canada) and incubated in ACK lysis buffer for 5 minutes to remove red blood cells. Cells were blocked with CD16/CD32 antibody (eBioscience, San Diego, CA, USA), and surfaced stained with alexa fluor 700, phycoerthrin, and PerCP conjugated anti-mouse CD3 (clone 17A2; eBioscience), NK1.1 (clone PK136; BD Pharmingen) and CD27 (clone LG.7F9, eBioscience) antibodies, respectively. Human PBMCs were isolated using Ficoll-Paque Plus (GE Healthcare) density-gradient centrifugation and surface stained using phycoerthrin and PerCP conjugated anti-human CD56 (clone B159; BD Pharmingen) and CD3 (clone UCHT1; BioLegend) antibodies, respectively. Stained cells fixed in 1% PFA were analyzed on a LSRII flow cytometer collecting 50,000 gated events and FlowJo flow cytometry analysis software.

3. Metastasis model

After treadmill acclimatization, high fat fed obese mice were injected intravenously with 5×10^5 murine mammary tumor virus/polyoma middle T (MT) cells. MT cells were grown in RPMI 1640 media (10% FBS, 1% penicillin/streptomycin, 1% L-glutamine, and 1% HEPES) and resuspended in sterile PBS upon injection. Lungs and livers were harvested and fixed in 2% paraformaldehyde for 48 hours. Tissue cross sections stained with H&E were photographed with a Leica microscope under 10× and 20× objectives.

4. Statistical analyses

Multiple group comparisons were assessed with a paired Student *t*-test or one-way ANOVA, followed by Tukey's post hoc multiple comparisons test using Graph Pad Prism 6 software (Graphpad Software Inc., La Jolla, CA, USA). Data are represented as mean ± SEM with significance indicated when $P < 0.05$.

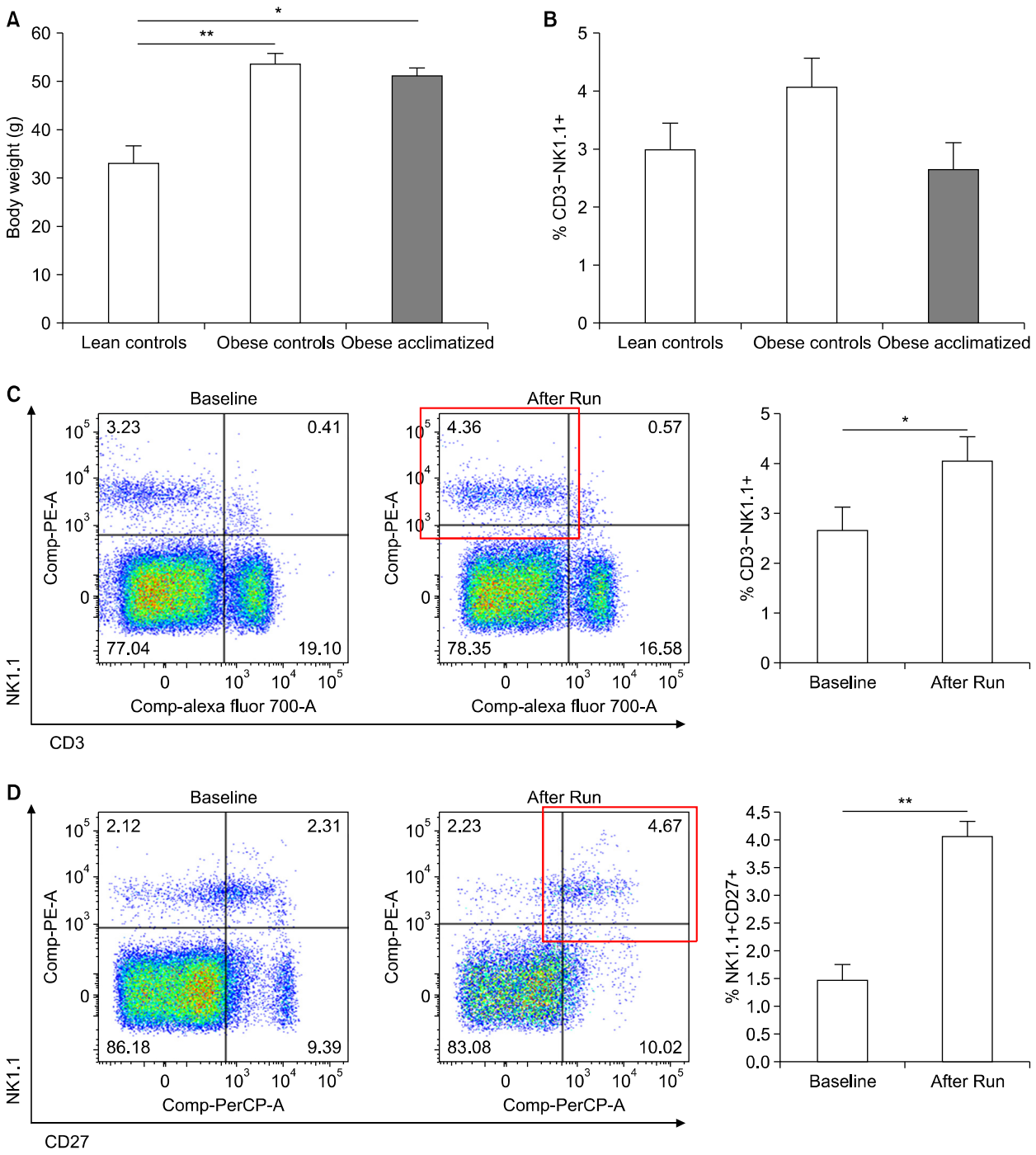

Figure 1. High intensity interval training (HIIT) increases the number and activity of circulating natural killer (NK) cells in obese female mice. C57Bl/6 (B6) mice were fed a 60% high fat in the obese groups or a chow diet in the lean control group. The obese groups either were acclimatized and then assigned to HIIT, or remained sedentary. The 4 weeks HIIT protocol involved 15 m/min treadmill running for 2 minutes followed by 2 minutes of rest, for a total of 60 minutes 3 d/wk. (A) The body weights of the mice. (B) Baseline and post-HIIT blood samples were collected, stained with anti-mouse CD3 and NK1.1 antibodies, and subsequently analyzed via flow cytometry. Baseline NK cell levels were compared between the groups in a bar graph. Shown are representative flow plots and bar graphs of the percentage increase in (C) circulating NK cell number (CD3−NK1.1+) and (D) activation (CD3−NK1.1+CD27+) in the HIT-acclimatized obese mice (n = 4-5 per group). $*P < 0.01$, $**P < 0.001$.

RESULTS

We employed a diet-induced obese mouse model to study HIIT's effects on circulating NK cells. While lean mice weighed significantly less than obese mice (Fig. 1A), flow cytometric analyses showed no difference in circulating baseline NK cell counts (CD3−NK1.1+) between groups (Fig. 1B). However, exercised obese mice had a significant increase in circulating NK cell number (Fig. 1C) and activation (Fig. 1D) compared to obese control mice.

To determine whether HIIT-induced elevation in NK cell count and activity affected metastasis, HIIT and sedentary obese mice were challenged with MT cells and lung tumor burden was assessed histologically. Tissue lung sections revealed that obese mice assigned to HIIT had remarkably less tumor burden compared to obese controls (Fig. 2).

Despite a dramatic reduction in tumor burden, HIIT did not induce weight loss in obese mice. To determine whether HIIT had any profound metabolic effect independent of weight loss, we analyzed histological liver cross sections and found a notable reduction in hepatic lipids in exercised obese mice compared to controls (Fig. 3).

To examine whether an acute bout of HIIT increases NK cells in human subjects, blood samples from overweight/obese female participants were analyzed for NK cell count and activation. In all three females, the percentage of circulating CD56+CD3−NK cells were markedly elevated after 20 minutes of high intensity exercise (Fig. 4A). We showed no significant effects of HIIT on other cell populations, including the CD3+ population which remained fairly unaffected post-HIIT (Fig. 4B).

DISCUSSION

Our results show that HIIT is an effective therapeutic strategy to enhance innate immunity in a mouse model of diet-induced obesity. In normal weight individuals, exercise-induced increase in circulating NK cells has been recognized for its therapeutic potential against breast cancer.[13,15] However, whether this also applies to the obese population has not been adequately investigated. Since increased adiposity exacerbates cancer burden and mortality, preventative and intervention strategies targeting obese individuals is warranted.[1] Our results show that HIIT can improve immunosurveillance by mobilizing NK cells and reduce tumor growth. Moreover, our findings validate the metabolic benefits of HIIT by reduced hepatic lipid deposition in obese mice. These observations suggest that HIIT may mitigate

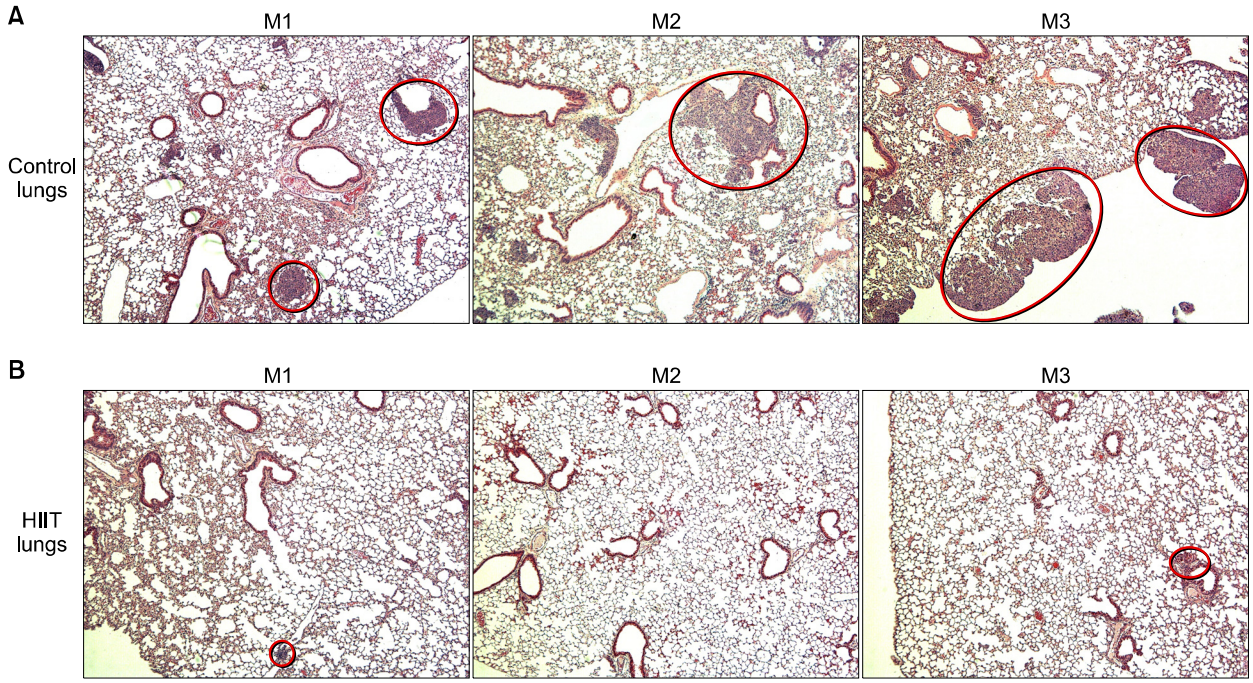

Figure 2. High intensity interval training (HIIT) reduces lung tumor burden in a metastasis obese mouse model. Obese mice were injected intravenously with 5×10^5 murine mammary tumor virus/polyoma middle T cells. Lungs were harvested and fixed in 2% paraformaldehyde for 48 hours. Tissue cross sections were stained with H&E and photographed under a 20× objective. Shown are representative histology slides of lung cross sections from (A) sedentary control and (B) HIIT mice (n = 3 per group). Circles indicate areas of tumors in the lungs.

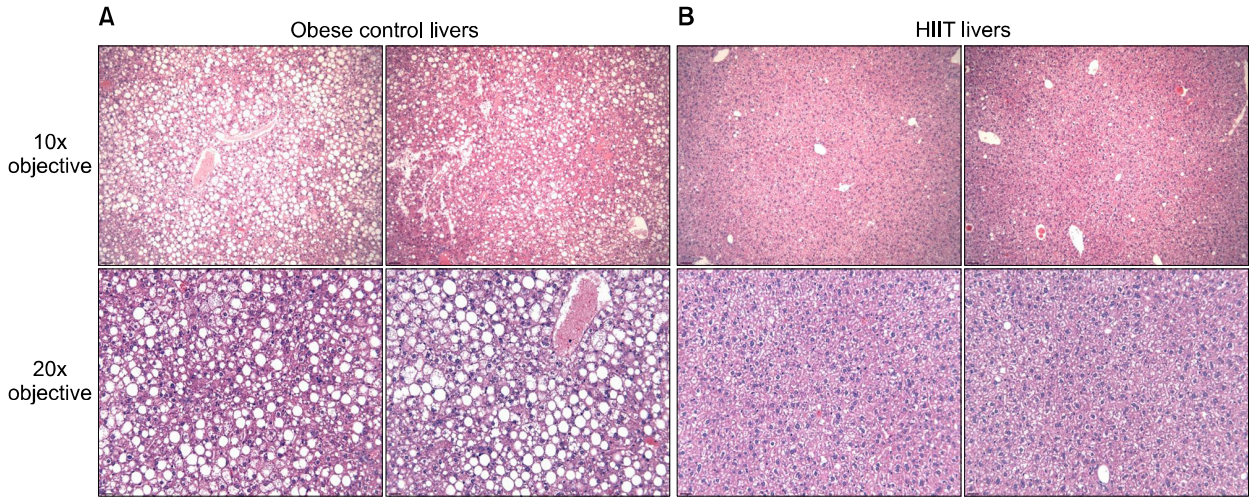

Figure 3. High intensity interval training (HIIT) reduces hepatic lipid deposition in obese mice. Livers were harvested and fixed in 2% paraformaldehyde for 48 hours. Tissue cross sections were stained with H&E and photographed under a 10× and 20× objective. Liver cross sections from (A) sedentary control and (B) HIIT mice were used to compare hepatic lipid content between the groups (n = 2 per group).

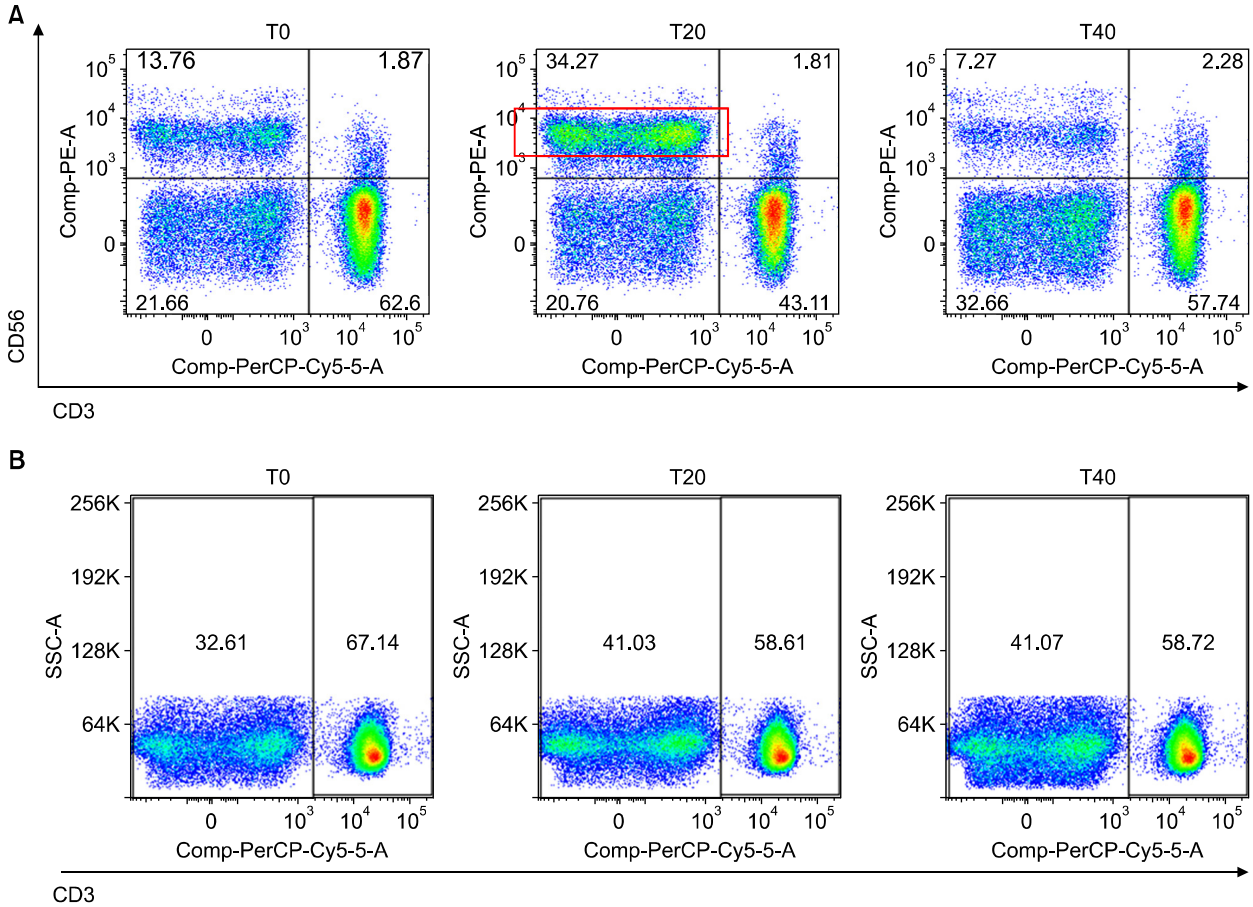

Figure 4. Circulating natural killer cell number and activation substantially increase by post-high intensity interval training (HITT) in overweight/obese women. Female participants were subjected to a 6 weeks HIIT protocol. Baseline and two post-HIIT blood samples were collected. Cells were stained with anti-human CD56 and CD3 antibodies and subsequently analyzed via flow cytometry. Shown are representative flow plots of (A) the percentage increase in circulating CD56+CD3− and (B) unaffected percentage of CD3-populations at baseline (T0), immediately post-HTIT (T20), and after a 20 minutes rest (T40) (n = 3).

the growth of obesity-associated cancer.

Increased expression of adipocyte-derived factors, such as leptin, promote breast tumorigenesis in obesity. Poor prognosis of breast cancer directly correlates with leptin levels and intratumoral leptin receptor expression in obese women.[18] Moreover, leptin treatment promotes proliferation in estrogen responsive breast tumor cells.[19] Interestingly, leptin has immunomodulatory effects. Diet-induced obesity results in the overexpression of suppressor of cytokine signalling 3, which desensitizes leptin receptor signalling in NK cells.[20] These leptin-resistant NK cells exhibit decreased stimulation.[20] Furthermore, long-term leptin exposure in obesity notably attenuates IFNγ production, proliferation, and cytotoxicity of NK cells.[21] Since obesity alters NK cell function, we attempted to determine whether exercise can stimulate NK cells in obese individuals to an extent similar in lean individuals. Of interest, exercise can decrease tumor-promoting adipokines, including leptin.[22] Our findings suggest that exercise may boost innate immunity through reducing leptin levels, as HIIT increased NK cell numbers in our obese breast cancer mouse model. This increase affirms the immunotherapeutic potential of HIIT against breast cancer in obesity.

The pathway from increasing circulating NK cells to resisting tumor development has been previously investigated. Following exercise, skeletal muscles release IL-15 and IL-6 into the bloodstream.[7,22,23] IL-6 sensitive NK cells are then mobilized through the binding of their β-adrenergic receptors by elevated epinephrine levels.[12,24] This mobilization is followed by increased intratumoral NK cell infiltration and tumor suppression.[24] Increased tumor infiltration may explain why circulating NK cell numbers dipped below basal levels in our female participants during the resting period. A previous study observed similar decreases in $CD56^{dim}$ cells as well as above-basal levels of the $CD56^{bright}$ subset.[25] While epinephrine elevates NK cell numbers, it does not increase the cytotoxicity on a per cell basis of this IL-6 sensitive population.[12,24] This suggests the role of IL-15 in activating NK cells. In obese patients, there are notably lower circulating IL-15 levels compared to lean individuals.[8] Low IL-15 levels are detrimental for NK cell development as IL-15 deficient mice lack peripheral NK cells.[8,26] This lack of NK cells directly correlated with extensive metastasis in IL-15 knockout mice challenged with breast tumors.[9] Physical activity may be an effective strategy to naturally upregulate IL-15, as one study showed increased circulating IL-15 levels in humans with post-exercise.[23] Upregulated IL-15 may be one factor explaining the observed increase in NK cell number and activity in our mouse and human models.

While exercise does not necessarily lead to weight loss, interval training has been shown to reduce body fat percentage in addition to other cardiometabolic improvements, including lower plasma glucose levels and maximal oxygen uptake.[27] Reducing body fat percentage may be an important regulator of obesity-derived inflammatory factors which promote tumorigenesis. In particular, a higher adipose tissue mass is known to increase basal lipolysis and therefore free fatty acids, as well as adipocyte necrosis. These events enhance macrophage infiltration in breast adipose tissue.[28] Moreover, cyclooxygenase enzymes can produce inflammatory prostaglandin E2 which suppresses NK cell activity and promotes tumourigenesis by acting on its G-protein coupled receptors.[29] Hence, lowering fat percentage through interval exercise may be critical in reducing pro-tumorigenic inflammation in obesity.

Here, we looked at the change in hepatic fat content between our obese mice. Obese mice undergoing HIIT exhibited a remarkable reduction in hepatic lipid deposition compared to control obese mice. Importantly, we observed no significant weight loss in these mice despite their decreased hepatic fat content. These results are consistent with previous findings exploring this exercise-induced decrease in hepatic fat, independent of weight loss.[30] In this study, we demonstrate how the benefits of HIIT in obese subjects are revealed through internal changes, including liver fat content, NK cell levels, and tumor resistance, rather than weight loss. Our findings signify how weight loss should not be viewed as an inclusive indicator of health in obese individuals, which is often a common misconception.

Using a mouse model of diet-induced obesity, HIIT increased the number of cytotoxic NK cells resulting in tumor resistance. This increase was also evidently detected in our female human participants undergoing HIIT. In addition to reducing tumor growth, HIIT reduces hepatic lipid content in obese mice, independent of changes in body weight. Altogether, these observations suggest that HIIT is a promising therapeutic option that can benefit obese breast cancer patients.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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